Testing for Variation in Animal Preference for Jesup Tall Fescue Hays with Wild-Type, Novel, or No Fungal Endophyte

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ABSTRACT

Tall fescue [Schedonorus phoenix (Scop.) Holub.] is an important source of forage but often contains a fungal endophyte [Neotyphodium coenophialum (Morgan-Jones and Gams) Glenn, Bacon, and Hanlin that can produce alkaloids and decrease animal performance. Removing the endophyte can reduce agronomic performance and persistence. An improved cultivar ('Jesup') with a novel (nontoxic) endophyte (trademark "MaxQ") has improved agronomic performance. Persistence may be related to agronomic traits and animal feeding preferences. We tested for variation in animal preference among two harvests by testing each of three hays made from Jesup tall fescue that varied in endophyte status (no endophyte, containing the novel endophyte, or containing a wild-type endophyte that produces alkaloids associated with toxicosis). We tested for preference using goats (Capra hircus), sheep (Ovis aries), and cattle (Bos taurus). All possible pairs were tested using traditional analysis of variance as well as multidimensional scaling. Multidimensional scaling of the animal preferences indicated that two dimensions were being used to rank the hays. The first dimension for all three animal species was related to variation in forage nutritive value. The second dimension was not correlated with any of the measured variables. Endophyte status was not a factor in the preferences expressed by the three animal species. It appears that no effective feedback mechanism exists related to endophyte status.

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Abbreviations: ADF, acid detergent fiber; ADIA, acid detergent insoluble ash; BW, body weight; CP, crude protein; DM, dry matter; IVTD, in vitro true dry matter disappearance; MDS, multidimensional scaling; NDF, neutral detergent fiber.

Tall fescue [Schedonorus phoenix (Scop.) Holub.] is an important forage resource across the North–South transition zone (Burns and Chamblee, 1979). In the upper South, tall fescue provides forage for pasture in late winter through spring and in the fall. Tall fescue is also an important source of hay with harvests generally occurring in late April through June.

Improved persistence of tall fescue along the southern portion of its range of adaptation has been attributed to the presence of the endophyte *Neotyphodium coenophialum* (Morgan-Jones and Gams) Glenn, Bacon, and Hanlin (Bacon et al., 1986; Bacon, 1995; Bouton et al., 1993, 2002; Porter, 1995). The same endophyte has been associated with tall fescue toxicosis (Hill et al., 2002) and reductions in animal weight gains (Fribourg et al., 1991; Schmidt and Osborn, 1993; Stuedemann and Hoveland, 1988). Reductions in animal gains have generally been attributed to interactions between the ergot alkaloids produced by the endophyte and the animal's physiological processes resulting in reduced daily dry

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matter (DM) intake and/or DM digestion (Aldrich et al., 1993a, 1993b; Burke et al., 2001a, 2001b; Emile et al., 2000; Fiorito et al., 1991; Hannah et al., 1990; Rice et al., 1997; Strickland et al., 1993).

The physiological response of ruminants to ergot-like alkaloids varies as a result of ambient temperature, concentration and type of endophyte consumed, the type and quantity of ergot-like alkaloids consumed, and individual animal physiology. Nutritive value of tall fescue, however, has shown little response to the presence of the endophyte with few differences observed between endophyte-free and toxic endophyte-infected forage (Asay et al., 2002; Collins, 1991; Burns and Fisher, 2006; Turner et al., 1990).

In the mid-Atlantic region, tall fescue hay is fed generally in the late fall through the midwinter season when day-time high temperatures seldom exceed 13°C. The tall fescue cultivar Jesup was developed and released for improved persistence in the mid-Atlantic region (Bouton et al., 1993). A novel (nontoxic) endophyte was incorporated into Jesup and is currently marketed under the trademark "MaxQ" tall fescue.

Variation in animal preference as a result of negative postingestive consequences may alter animal feeding behavior. No data are available to indicate whether animals can detect negative postingestive (short-term) feedback from consumption of endophytes or alkaloids. Preference for tall fescue with no endophyte or with a novel endophyte such as MaxQ may interact with hay or pasture feeding management, with consequences for animal performance and plant persistence.

The objectives of this study were to test for variation in relative preference, expressed as short-term intake, among two harvests of three Jesup tall fescue hays containing either no endophyte, a novel endophyte (MaxQ), or a wild-type endophyte in goats (*Capra hircus*), sheep (*Ovis aries*), and cattle (*Bos taurus*).

MATERIALS AND METHODS

Experimental Hays

The experimental hays were harvested from well-established stands of Jesup tall fescue grown on a Cecil clay loam (fine, kaolinitic, thermic Typic Kanhapludult) soil at the North Carolina State University Reedy Creek Road Field Laboratory near Raleigh, NC. Three treatments were cut for evaluation at two harvest dates. These consisted of endophyte-free Jesup, Jesup with an endophyte considered nontoxic and marketed under the trademark MaxQ, and Jesup with a wild-type endophyte.

All stands of tall fescue were flail chopped to an 8-cm stubble in late February to remove all winter carry-over growth. The areas were limed and top-dressed with P and K according to soil test. Ammonium nitrate was top-dressed in early March and again after the removal of the initial growth at 78 kg N ha⁻¹ per application. Initial growth was cut in the midboot stage on 20 Apr. 2001 (Harvest 1). Regrowth was cut 29 June 2001 when canopy height reached about 30 cm and was vegetative (Harvest 2). Growing

conditions were near typical with Harvest 1 produced under cooler temperatures than Harvest 2 (Burns and Fisher, 2006).

All forage was cut with a mower conditioner set to cut at 10 cm. After cutting, and again each day until baling, the forage was redistributed with a tedder to aid drying. The forage was then baled with a conventional square baler and stored on wooden pallets as experimental hays in a metal building until fed.

All treatments were field cured without exposure to rain and were preserved without heat damage. Endophyte infection averaged 94.0, 95.3, and 5.3% for the wild-type endophyte, novel endophyte, and endophyte-free treatments, respectively (Burns and Fisher, 2006). In a previously reported study, repeated sampling of these fields over 2 yr with three sampling dates per year indicated an average ergovaline content of 221 µg kg⁻¹ in the wild-type tall fescue compared to a mean of 33 $\mu g \ kg^{-1}$ for the nontoxic endophyte and the endophyte-free samples (Burns et al., 2006). The nontoxic and the endophyte-free samples were similar. Use of an ELISA ergot alkaloid immunoassay on samples of the hays produced for this trial resulted in a response in the wild-type endophyte hay that was approximately twice as great as the background response in the nontoxic endophyte or endophyte-free treatments (Hill et al., 2000). No difference was found between the two harvests or between the endophyte-free and the nontoxic endophyte treatments.

Just before feeding, the hays were passed through a hydraulic bale processor (Van Dale 5600, J. Starr Industries, Fort Atkinson, WI) with stationary knives spaced at 10 cm. The processed hays were cut into lengths of 7 to 13 cm. This procedure is used to reduce losses during feeding.

Design of Preference Trials

We conducted three experiments that used different animal species to test for variation in preference using methodology previously shown to be effective at detecting small differences in preference among fescue hays (Fisher et al., 1999). In the first experiment, six Spanish doe goats were used (mean body weight [BW] = 38.8 kg, SD = 1.4); in the second experiment, six Katahdin ewe sheep were used (mean BW = 42.3 kg, SD = 2.0); and in the third experiment, six Hereford steer cattle were used (mean BW = 436 kg, SD = 233). The protocol for animal care and health was approved by the North Carolina State University Institutional Animal Care and Use Committee.

During an adaptation or training period (Kyriazakis et al., 1990; Fisher et al., 1999), meals of each of the hays were offered to allow the animals to associate the hay with postingestive metabolic feedbacks and taste produced by the forage (Villalba et al., 2006). This training period lasted 6 d and we randomized the order in which the forages were offered to each animal.

Since we were testing six experimental hays we had 15 pairs to examine. After the training period and during the experimental phase, we presented each possible pair of hays for a meal in the morning to test for preference. Only one pair was offered each day. The experimental phase lasted 15 d. The order of presentation of the pairs and the left-right position of the hays in the pair were randomized. The weight of hay was determined before and after feeding. This permitted calculation of DM consumed after adjusting for the DM concentration of the hay.

Animals were individually penned in all three experiments. Sheep and goat pens were approximately 1.5 by 2 m. Cattle

pens were approximately 2.5 by 4 m. We presented the pair of forages side by side with sheep and goats offered approximately 0.75 kg of each hay and allowed 2.5 h for the meal.

The cattle were led into the pens, offered approximately 2 kg of each hay, and allowed 30 min to feed. Only two pens were available for cattle so approximately 2 h were required to finish evaluation of each morning's hay pairs. Cattle were housed and fed in stalls for the remainder of the day. For the experiment with cattle, a video recorder was used to estimate the total time spent at each feeder to calculate intake rate by dividing hay disappearance by minutes at the feeder.

In all three experiments we took care to keep sufficient forage of both treatments available during the trial so that each animal always had a choice between the two hays being tested. Each day, after the preference trial, animals were given ad libitum access to a hay not included in the trial.

Laboratory Analyses

In each of the three experiments (goat, sheep, and cattle) forage samples were analyzed that were comprised of subsamples collected each time a hay was fed in a pair (n = 5). Samples were then composited for each animal and represented the forage offered to each animal. This subsample included variation within the hay on offer as well as laboratory variation in our estimates of means (n = 6). The composite sample was dried at 75°C in a forced draft oven and composition values were reported on a DM basis. Samples were ground to pass a 1-mm screen in a cyclone mill.

In vitro true DM disappearance (IVTD) was determined using ruminal inoculum collected from a cannulated mature Hereford steer fed a mixed alfalfa (*Medicago sativa* L.) and orchardgrass (*Dactylis glomerata* L.) hay. After incubation for 48 h with ruminal inoculum (Ankom Technology Corp., Fairport, NY) samples were extracted with neutral detergent solution for estimation of IVTD.

Neutral detergent fiber (NDF), acid detergent fiber (ADF), cellulose, acid detergent lignin, and acid detergent insoluble ash (ADIA) were estimated according to Van Soest and Robertson (1980) in a batch processor (Ankom Technology Corp., Fairport, NY) for samples of the hays. Crude protein (CP) was estimated as 6.25 times the percentage of N determined with an Auto Analyzer (Technicon Industrial Systems, Tarrytown, NY) for both masticate and hay samples (Association of Official Analytical Chemists, 1990).

All samples were scanned for near-infrared reflectance spectroscopy and the observed values were used to develop prediction equations. The prediction equations were then used to estimate each observation (Table 1).

Statistical Analysis

The experimental design allowed statistical analysis by multidimensional scaling (MDS) as well as by traditional analyses (Buntinx et al., 1997; Fisher et al., 1999, 2002). Multidimensional scaling is used to develop a spatial arrangement representing the differences expressed as selective forage intake presented in pairs to the animals. For MDS, the difference in preference between a pair of hays was expressed by subtracting the amount of the least preferred hay from the most preferred hay and dividing by the sum of the two intakes. In this way, preference was expressed numerically on a scale

from 0 to 1. For example, if the animal consumed equal quantities of the hays in the pair, then the difference ratio is equal to 0 and no preference is expressed. If only one of the pair was consumed then the difference ratio is equal to 1 and the maximum difference in preference between hays is expressed (Buntinx et al., 1997; Fisher et al., 1999, 2002). These means and variance of these estimated differences are scaled as they are analyzed in MDS and therefore the MDS dimensions may be >1.

In addition to MDS, each experiment was tested by analysis of variance after averaging intake of each hay (averaged across each combination, n = 5) by each animal. The analysis of variance included terms for animal and hay and means were tested with orthogonal contrasts.

Simple linear correlation was used to examine the relationship of DM intake to nutritive value.

RESULTS

Goat Responses (Experiment 1)

Goats preferred hay from the first harvest over the second harvest (Table 2). This preference may be explained by the large differences in nutritive value between the two

Table 1. The ranges of each estimate of nutritive value predicted by near infrared reflectance spectrophotometer, its SE of calibration (SEC) and SE of cross validation (SEV).

Variable [†]	N	Range	SEC	SEV	Mean
			g k	(g ⁻¹	
NDF	112	564-706	7.3	8.5	627
ADF	115	273-405	4.6	5.9	310
CELL	115	232-349	3.5	4.4	271
Lignin	111	21.1-54.8	1.9	2.1	32
IVTD	115	619-833	10.9	12.9	745
CP	114	105-193	2.1	2.7	148

[†]NDF, neutral detergent fiber; ADF, acid detergent fiber; CELL, cellulose; IVTD, in vitro true dry matter disappearance; CP, crude protein.

Table 2. Intake and composition of two harvests (H1 and H2) of fescue hay with either the wild-type (W) or novel endophyte (N) or free of the endophyte (F) fed to goats (Experiment 1).[†]

Forage	Intake [‡]	NDF	ADF	Cell	Hemi	Lignin	ADIA	IVTD	СР
	g				——g	kg-1			
H1-W	208	591	290	259	301	25	4.8	810	164
H1-N	218	580	286	256	295	24	4.8	812	162
H1-F	250	573	282	251	291	24	5.1	820	166
H2-W	21	643	319	288	324	26	5.4	731	134
H2-N	27	643	316	286	327	26	5.7	729	120
H2-F	38	645	331	294	313	32	5.4	719	128
Contrasts		Res	ults of	ortho	gonal	contra	sts (P >	> F)	
H1 vs. H2	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
W vs. N	0.56	< 0.01	< 0.01	<0.01	0.18	0.11	0.08	0.96	< 0.01
W vs. F	0.04	< 0.01	0.03	0.28	< 0.01	< 0.01	0.17	0.51	0.10
H × (W vs. N)	0.85	< 0.01	0.56	0.91	< 0.01	0.01	0.20	0.22	<0.01
$H \times (W \text{ vs. F})$	0.36	< 0.01	<0.01	< 0.01	0.61	< 0.01	0.29	< 0.01	0.01

[†]NDF, neutral detergent fiber; ADF, acid detergent fiber; Cell, cellulose; Hemi, hemicellulose; Lignin, sulfuric acid lignin; ADIA, acid detergent insoluble ash; IVTD, in vitro true dry matter disappearance; CP, crude protein.

[‡]Values are means of six observations during feeding of six animals.

harvests. The preferred harvest had lower NDF, ADF, cellulose, hemicellulose, lignin, and ADIA and greater IVTD and CP. Goats also preferred the endophyte-free tall fescue over tall fescue with the wild-type endophyte. However, this preference may have been associated with greater nutritive value of the endophyte-free fescue in the first harvest. Estimates of nutritive value interacted between harvest and the wild-type and endophyte-free tall fescue for NDF, ADF, cellulose, lignin, IVTD, and CP. Nutritive value does not provide an explanation of the preference for endophyte-free tall fescue over the wild-type endophyte tall fescue in the second harvest. All three fescue hays from this harvest were much less palatable than hays from the first harvest (Table 2). Multidimensional scaling in two dimensions separated the hay treatments in the first dimension (Dim 1) by a variable that appears associated with harvest (Fig. 1). The second dimension (Dim 2) indicates that the goats had other criteria that were important after the first dimension for selection within a harvest.

Reduced nutritive value between the first and second harvest was evident in the increased NDF, ADF, cellulose, hemicellulose, lignin, and ADIA and reduced IVTD and CP of the second harvest (Table 2). Numerous interactions between harvest and endophyte status were detected. For example, harvest interacted with tests of wild-type endophyte versus endophyte-free fescue for six of eight measures of nutritive value. Harvest also interacted with tests of wild-type endophyte by novel endophyte for four of eight measures of nutritive value. The interactions were likely a result of slight differences in rates of maturity in early spring and summer associated with endophyte status.

The interactions in nutritive value were not reflected in intake during the preference trials. For example, IVTD interacted across harvests for the wild-type endophyte fescue and the endophyte-free tall fescue but intake did not interact with harvest. The precision of the estimates of nutritive value results in significant differences that are small but these differences are similar in magnitude to those associated with preference in other trials (Fisher et al., 1999, 2002).

Sheep Responses (Experiment 2)

Sheep also preferred the first harvest over the second harvest reflecting the greater overall nutritive value of the first harvest (Table 3). In contrast to the goats, we found that harvest interacted in orthogonal contrasts for wild-type endophyte fescue versus the novel type endophyte and wild-type endophyte versus the endophyte-free fescue. In the first harvest, the endophyte-free fescue and the fescue with the novel endophyte were preferred over the fescue with the wild-type endophyte; however, in the second harvest fescue with the wild-type endophyte was preferred over either of the other plant materials. Multidimensional scaling results contrasted between the goats and sheep (Fig.

1). Sheep separated both harvests in two dimensions while goats clustered the first harvest with little separation.

Although the estimates of nutritive value were based on samples collected separately during the sheep trial, they are

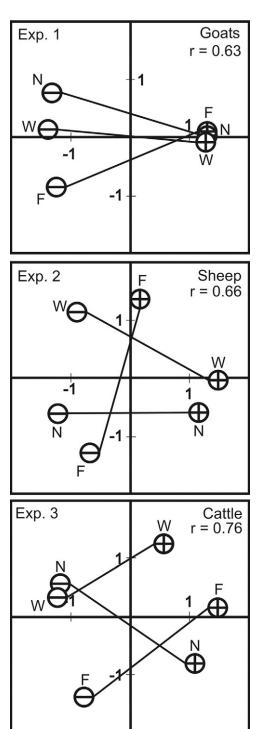


Figure 1. Results of multidimensional scaling of preference observations from three experiments with more preferred forages generally located closer to the upper right corner and less preferred forages generally located closer to the lower left corner. Each pair of hays representing an endophyte status is linked with a line (F, endophyte free; W, wild-type endophyte; N, novel endophyte). Circles with plus signs indicate hays with greater nutritive value (Harvest 1) while circles with minus signs indicate hays with lesser nutritive value (Harvest 2).

naturally similar to the estimates collected during the goat trial. The interactions observed in the estimates of nutritive value are more consistent with the observed variation in intake in the sheep experiment. For example, the preference for the fescue with the wild-type endophyte in the second harvest may be related to the generally greater nutritive value of the wild-type endophyte fescue over the other two types in the second harvest. The significant interactions of the contrasts for IVTD and CP with harvest may partially explain the variation observed in intake. In the second harvest the IVTD and CP increased in the fescue containing the wild-type endophyte relative to the two other fescues. The estimates of fiber concentration also interacted with harvest. Compared across harvests, NDF, hemicellulose, and lignin fractions differed between tall fescue with the

wild-type and novel endophytes, but these interactions were the result of relatively small differences. Interactions were observed for all fiber fractions except hemicellulose when tall fescue with wild-type endophyte was compared with endophyte-free tall fescue. The endophyte-free fescue had more NDF, ADF, cellulose, and lignin than the fescue with the wild-type endophyte in the second harvest. These interactions across harvests are consistent with the variation observed in the IVTD and CP and may partially explain the variation observed in intake.

Steer Responses (Experiment 3)

As was found with goats and sheep, steers preferred the first harvest over the second harvest but intake did not show as pronounced a preference between the two harvests (Table 4).

Table 3. Intake and composition of two harvests (H1 and H2) of fescue hay with either the wild-type (W) or novel endophyte (N) or free of the endophyte (F) fed to sheep (Exp. 2).

Forage	Intake [‡]	NDF	ADF	Cell	Hemi	Lignin	ADIA	IVTD	СР	
	g		g kg ⁻¹							
H1-W	326	586	284	254	302	23	4.6	818	165	
H1-N	388	576	279	251	297	22	4.8	823	162	
H1–F	412	570	280	249	289	24	5.1	817	165	
H2-W	44	636	315	284	321	25	5.1	741	135	
H2-N	23	639	313	282	327	26	5.7	728	120	
H2-F	24	640	328	291	312	32	5.5	716	126	
Contrast				Results of or	thogonal co	ntrasts (P > F)			
H1 vs. H2	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	
W vs. N	0.29	0.04	0.01	0.02	0.77	0.71	0.01	0.11	< 0.01	
W vs. F	0.09	< 0.01	< 0.01	0.74	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	
$H \times (W \text{ vs. } N)$	0.04	< 0.01	0.49	0.79	< 0.01	0.03	0.16	< 0.01	< 0.01	
$H \times (W \text{ vs. } F)$	< 0.01	< 0.01	< 0.01	< 0.01	0.10	< 0.01	0.71	< 0.01	< 0.01	

[†]NDF, neutral detergent fiber; ADF, acid detergent fiber; Cell, cellulose; Hemi, hemicellulose; Lignin, sulfuric acid lignin; ADIA, acid detergent insoluble ash; IVTD, in vitro true dry matter disappearance; CP, crude protein.

Table 4. Intake, intake rate (Int. rate), and composition of two harvests (H1 and H2) of fescue hay with either the wild-type (W) or novel endophyte (N) or free of the endophyte (F) fed to cattle (Exp. 3).[†]

Forage	Intake [‡]	Int. rate	NDF	ADF	Cell	Hemi	Lignin	ADIA	IVTD	СР
	g	g min ⁻¹		g kg ⁻¹						
H1-W	463	49	572	277	246	295	25	5.4	805	170
H1-N	555	42	587	284	254	303	24	4.9	813	165
H1-F	723	53	570	283	251	287	24	4.7	818	162
H2-W	314	71	636	314	280	322	27	4.9	731	145
H2-N	317	46	637	310	279	327	25	4.7	743	134
H2-F	238	40	634	318	280	315	31	4.5	733	147
Contrast				Results	of orthogo	nal contrast	ts (P > F)			
H1 vs. H2	< 0.01	0.61	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
W vs. N	0.62	0.16	< 0.01	0.25	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
W vs. F	0.34	0.26	0.13	< 0.01	0.01	< 0.01	< 0.01	< 0.01	< 0.01	0.01
$H \times (W \text{ vs. } N)$	0.64	0.43	< 0.01	< 0.01	< 0.01	0.93	0.02	0.18	0.29	0.01
$H \times (W \text{ vs. } F)$	0.09	0.12	0.88	0.61	< 0.01	0.10	< 0.01	0.24	0.33	< 0.01

[†]NDF, neutral detergent fiber; ADF, acid detergent fiber; Cell, cellulose; Hemi, hemicellulose; Lignin, sulfuric acid lignin; ADIA, acid detergent insoluble ash; IVTD, in vitro true dry matter disappearance; CP, crude protein.

[‡]Values are means of six observations during feeding of six animals.

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Table 5. Correlations (r) and probabilities of a greater correlation (P > r) for dry matter intake (DMI), dry matter intake rate (DMIR), and dimensional coordinates from multidimensional scaling (Dim 1 and Dim 2) with estimates of nutritive value in Experiments 1, 2, and 3.[†]

Variable		NDF	ADF	Cell	Hemi	Lignin	ADIA	IVTD	CP		
Experiment 1											
Goat-DMI	r	-0.99	-0.96	-0.98	-0.96	-0.64	-0.86	0.99	0.97		
	p > r	< 0.01	< 0.01	< 0.01	< 0.01	0.17	0.03	< 0.01	< 0.01		
Goat-Dim 1	r	-0.99	-0.96	-0.98	-0.94	-0.65	-0.90	0.99	0.97		
	p > r	< 0.01	< 0.01	< 0.01	< 0.01	0.16	0.01	< 0.01	< 0.01		
Goat-Dim 2	r	-0.06	-0.27	-0.17	0.26	-0.70	0.24	0.10	-0.07		
	<i>p</i> > <i>r</i>	0.91	0.60	0.75	0.62	0.12	0.64	0.85	0.89		
				Experin	nent 2						
Sheep-DMI	r	-0.99	-0.97	-0.99	-0.93	-0.72	-0.75	0.98	0.97		
	<i>p</i> > <i>r</i>	< 0.01	0.01	< 0.01	0.01	0.11	0.08	< 0.01	< 0.01		
Sheep-Dim 1	r	-0.85	-0.83	-0.85	-0.76	-0.63	-0.90	0.90	0.90		
	p > r	0.03	0.04	0.03	0.08	0.18	0.01	0.02	0.02		
Sheep-Dim 2	r	-0.35	-0.38	-0.36	-0.28	-0.50	-0.32	0.36	0.41		
	<i>p</i> > <i>r</i>	0.50	0.46	0.48	0.59	0.32	0.54	0.49	0.42		
				Experin	nent 3						
Cattle-DMI	r	-0.88	-0.84	-0.83	-0.86	-0.75	0.21	0.92	0.70		
	p > r	0.02	0.04	0.04	0.03	0.09	0.69	0.01	0.12		
Cattle-DMIR	r	0.16	0.17	0.19	0.16	-0.09	0.22	-0.24	-0.15		
	<i>p</i> > <i>r</i>	0.76	0.74	0.72	0.77	0.86	0.68	0.64	0.77		
Cattle-Dim 1	r	-0.95	-0.90	-0.92	-0.94	-0.60	0.29	0.97	0.87		
	p > r	< 0.01	0.01	0.01	0.01	0.21	0.58	0.01	0.02		
Cattle-Dim 2	r	-0.27	-0.37	-0.32	-0.13	-0.54	0.70	0.19	0.11		
	<i>p</i> > <i>r</i>	0.60	0.47	0.54	0.81	0.26	0.12	0.72	0.83		

[†]NDF, neutral detergent fiber; ADF, acid detergent fiber; Cell, cellulose; Hemi, hemicellulose; Lignin, sulfuric acid lignin; ADIA, acid detergent insoluble ash; IVTD, in vitro true dry matter disappearance; CP, crude protein.

In contrast to the small ruminants, the steers did not demonstrate any preferences based on endophyte status either as a main effect or as an interaction with harvest. We may, however, have a Type II statistical error in the comparison of the interaction of harvest with the preference for the fescue with the wild-type endophyte versus the endophyte-free fescue (P = 0.09). The relative intakes of each pair produced relatively good fits during MDS (Fig. 1). The second harvest fescues with the wild-type and the novel type endophytes were judged to be similar and are plotted close to each other and that is consistent with the intake data (Table 4). The tabular data is single-dimensional and in the first harvest fails to indicate the difference in preference between the fescue with the wild-type endophyte and the fescue with the novel endophyte detected by the MDS procedure (Fig. 1). Calculating means can mask differences between individual pairs but MDS allows a spatial analysis that reveals differences among individual pairs. Multidimensional scaling showed that the steers expressed a difference in preference between the hay with the wild-type endophyte and the hay with the novel endophyte. The hay with the novel endophyte is greater in dimension 1 and MDS shows that the steers judged it to be more like the endophyte free hay in the two-dimensional space than the hay with the wild-type endophyte.

Correlations

Correlations (r) were examined to relate composition to intake and the MDS dimensions (Table 5). The orthogonal

MDS dimensions are statistical dimensions but they can be related to measured variables using correlation. Therefore, intake behavior was tested as DMI and also as the two orthogonal dimensions of MDS. Dry matter intake rate was also available for the experiment with cattle but was not correlated with any variable and showed a lack of significant effects in the analysis of variance (data not shown).

The DMI and the first MDS dimension were closely correlated with various measures of nutritive value in all three experiments. Across all three experiments NDF and IVTD were particularly well correlated with DMI and the first MDS dimension. In the MDS analysis, the first dimension accounts for most of the sum of squares and consequently the correlations between the explanatory variables and both the DMI and Dimension 1 from MDS are often similar. This effect can even result in a single MDS degree of freedom that is simply associated with nutritive value (Fisher et al., 1999).

In the three experiments reported here, the second MDS dimension was not correlated with any of the measured variables. One or more unknown variables are likely to be associated with this dimension and responsible for the small but significant interactions observed between harvest and endophyte status.

CONCLUSIONS

All three animal species responded to variation in fiber and digestibility among the six hays with varied preferences.

This behavior was similar to other studies in which animals showed an ability to detect feed with less fiber and greater digestibility, remember the feeds at a later meal, and consume more of the feed with the greater nutritive value when presented in pairs. Both mean intake and the first MDS dimension demonstrated this behavior. The second dimension from MDS was not associated with any of the measured variables and may have been associated with a taste or aroma since it is unlikely that a texture variable was involved. Animals did not express a preference for one endophyte status over another. This may partially explain why animals do not reduce feed intake before the onset of severe toxicosis.

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